ClinicalTrials.gov Study Document

Official Study Title: The O2-PRBC.Thal study on the use of Hemanext One RBC Storage System for blood transfusion support in transfusion-dependent thalassemia

NCT Number: Pending

Document Type: Study Protocol and Statistical Analysis Plan Document

Date: NOV 2025

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Introduction

Patients with transfusion dependence thalassemia (TDT) are regularly transfused every two to four weeks. Their long-term survival and quality of health is dependent on the quality of the transfused blood. TDT patients experience significant problems and organ dysfunction due to chronic anemia, chronic iron overload as well as long life use of different medications, like iron chelators. TDT patients usually receive 2-4 units of red blood cells (RBC) (each unit: 250300ml), per month.

RBCs storage processing aims to preserve the RBCs' properties. Long-term storage leads to donor-dependent biochemical and morphological changes in RBCs collectively termed as "storage lesions". Transfusion of stored RBCs for more than 1 to 2 weeks is associated with increased post-transfusion mortality rates [1-4]. Therefore, there is a need for developing optimized processing and storage conditions. Considering the primary functional role of RBCs, which is oxygen transport and exchange, focusing on these processes might be a potential way for improving storage quality [1,5].

RBCs' "storage lesions" have recently been correlated with metabolic reprogramming [6]. Dysregulation of RBCs' homeostasis disrupts structural lipids and proteins, resulting in progressive pathological alterations in RBC morphology, and downregulates RBCs' survival post-transfusion [6]. "Storage lesions" represent a wide spectrum of RBCs' deformities being associated with oxidative damage, manifesting as post-translational changes in proteins (e.g., hemoglobin, peroxiredoxin 2, anion exchanger 1, catalase, pyruvate kinase, glucose 6phosphate dehydrogenase) and depletion of redox metabolites (e.g., glutathione) [7-13]. Glycolytic enzymes, especially glyceraldehyde 3-phosphate dehydrogenase (GADPH), have been shown *in vitro* to mediate this process; oxygen is considered to modulate the competitive binding of glycolytic enzymes and deoxyhemoglobin with N-terminal cytosolic domains [14]. Specifically, GADPH, is linked to the depletion of 2,3-diphosphoglycerate (2,3-DPG) during the first 2 weeks of storage [3,9,15-17]. Oxygen saturation levels are considered to modulate the competitive binding of GADPH and deoxyhemoglobin with N-terminal cytosolic domains [14]. Under pro-oxidant conditions, hemoglobin turns into its oxygenated substance decreasing its binding affinity, while GADPH, migrates to binding sited of the nearby structural proteins. Ectopic localization of GADPH to the membrane and binding with structural proteins, has been associated with loss of activity, blocking glycolysis, and causing various morphologic and functionality defects on the binding proteins [14].

Studies have shown that RBCs stored under hypoxic conditions preserved faster O_2 kinetics compared with the standard storage protocols, with this difference being reported in the first 35 days of storage; that is, the longest permissible shelf-life of blood in the United Kingdom and many European countries [1]. In clinical practice, the median storage duration of transfused units is 21 days. The effects on O_2 after RBCs' storage in hypoxic conditions are similar to the ones of biochemical rejuvenation following standard storage – however RBCs' storage in hypoxic conditions presents advantages in terms of easier clinical use, as it can be conducted on units in bulk and before dispatch to hospitals [1].

The **Hemanext ONE RBC Processing and Storage system** limits oxygen, the fuel for oxidative damage, thus, providing a higher quality RBCs. It has the potential to benefit all patients requiring transfusion for chronic conditions such as thalassemia, sickle cell disease (SCD), and myelodysplastic syndromes (MDS), as well as those in need of critical transfusions during post-traumatic surgery and other medical procedures [18-26]. Hemanext ONE creates hypoxic RBCs, RBCs that have been processed to reduce oxygen and carbon dioxide content of RBCs and to maintain this level throughout storage up to 42 days. Hypoxic RBCs have demonstrated positive impacts on multiple in vitro metrics of RBC quality in preclinical studies. Clinical studies are underway to determine the impact of hypoxic RBCs on patient outcomes and estimate potential cost savings from expected improvements in care and reductions in transfusion volumes.
Hemanext ONE has been granted marketing authorization for commercial distribution via the De Novo process by the U.S. Food & Drug Administration. In Europe, Hemanext ONE is CE marked which allows the medical device to be placed in the market in the European Economic Area (EEA).
Hemanext ONE has not been studied in chronically transfused patients like thalassemia.
It is imperative to establish the safety and the efficacy, especially in regard to required quantity of blood transfusion prepared with the Hemanext ONE processing, before its general application to sensitive groups of patients like TDT. In this respect, there are many issues to be investigated, like long-term biochemical results and changes in the primary physiological function of RBCs in thalassemic recipients.

The aim of our study is to investigate whether RBCs' storage under hypoxic conditions is at least non inferior to standard conditions in providing transfusion support to TDT patients without increasing the transfusion burden, the degree of hemolysis and of metabolic disturbances, and the rate of iron and erythropoiesis, while improving our patients' quality of life (QOL).

Objectives

- To validate the feasibility of applying the Hemanext ONE processing and storage of blood units in the routine care of transfusion dependent thalassemic (TDT) patients
- To evaluate the safety of Hemanext ONE in TDT
- To evaluate the changes in transfusion burden when using the Hemanext ONE system
- To evaluate changes in surrogate markers of metabolism, hemolysis, iron overload and erythropoiesis, when using the Hemanext ONE system
- To assess QOL changes

Hypothesis

The hypothesis is that using the hemanext one process is able to provide transfusion support to TDT patients without increasing the transfusion burden, the degree of hemolysis and metabolic disturbances, and the rate of iron and erythropoiesis.

Primary Endpoint

Changes from baseline in RBC transfusion burden. Significant changes are defined as a difference of >20% from baseline, corresponding to at least 1 unit/ 24weeks.

Secondary Endpoints

- Changes from baseline in pre-transfusion hemoglobin
- Changes on mean total Hb mass
- Changes from baseline in metabolic markers
- Changes from baseline in other markers of hemolysis and erythropoiesis
- Changes in QOL
- Overall changes from baseline in markers of iron overload
- Type, frequency, severity, seriousness of AEs and relationship of AEs to Hemanext. AEs of interest include hemolytic reactions and

METHODOLOGY

Patients

TDT patients followed at the Thalassemia Unit either of the First Dept of Pediatrics NKUA or at the LAIKON General Hospital and fulfilling the following criteria will be invited to participate in the study.

Inclusion Criteria

Subjects must satisfy the following criteria to be enrolled in the study:

1. Subject is ≥ 18 years of age at the time of signing the informed consent form (ICF).
2. Subject must understand and voluntarily sign an ICF prior to any study-related assessments/procedures being conducted.
3. Subject has documented diagnosis of β- transfusion dependent thalassemia, defined as ect: ≥ 6 RBC units/24 weeks and no transfusion-free period for ≥ 42 days during the 24 weeks prior enrollment.
4. Transfusion history (including units or cc) for at least 6 months prior to enrollment needs to be available.
5. Subject is on chelation therapy and a fairly stable dose for at least 6 months prior to enrollment.
6. Chronic therapies (including hydroxyurea) are allowed as long as the medication dose has been stable for at least 6 months prior to enrollment.
7. Having been exposed to PRBC transfusions prepared with the Hemanext One processing system

Exclusion Criteria

The presence of any of the following will exclude a subject from enrollment:

1. Subject has currently or has a history of any significant medical condition, laboratory abnormality, or psychiatric illness.
2. Subject has had positive Coombs (antiglobulin) test anytime during the 6 months prior to enrollment.
3. Subject is scheduled to undergo or has recently (in the last 6 months) undergone splenectomy.
4. Use of luspatercept during the period of 6 months prior to enrollment and during the trial is not allowed.

5. Participation in an interventional clinical trial or use of experimental medications during the period of 6 months prior to enrollment and during the trial is not allowed.

Methods

The trial will consist of two phases.

- Baseline phase (retrospective or prospective)

The baseline phase consists of 12 weeks, evaluated retrospectively or prospectively, just prior or after receiving Hemanext transfusion. During this phase, the patients are expected to have received packed red blood cells (PRBC) processed using standard method. The phase will be used to establish their baseline transfusion and hematologic characteristics. Baseline values will be derived from this phase.

- Treatment Phase

The treatment phase will last for at least 3 months, during which patients will continue receiving PRBCs prepared using the Hemanext methodology. This phase will continue for as long as the patient receives Hemanext-prepared PRBC transfusions and may be extended until the data cut-off date. The data cut-off date is defined as the time point when data have been collected for at least 3 months from all patients included in the study. Each patient must sign the Informed Consent Form before any data collection begins.

HEMANEXT ONE process and store CP2D/AS-3 Red Blood Cells, Leukocytes Reduced (LR RBC) that have been prepared within the standard 8-hour hold time. Processing must be initiated within 8 hours of collection and completed within 12 hours of collection. The Red Blood Cells must be processed at room temperature (20-26°C). The HEMANEXT ONE system limits O_2 and CO_2 levels in the storage environment. Red Blood Cells Leukocytes Reduced, O_2/ CO_2 Reduced may be stored for up to 42 days at 1-6°C. HEMANEXT ONE is used for volumes no greater than 350 mL of LR RBC.

Transfusion Burden

As this is the primary end point, the transfusion burden should be calculated based on cc/kg. Number of blood units could also be registered and if available hematocrit of the PRBC's. Baseline values will be based on the baseline phase.

Transfusion Efficacy

To evaluate further the efficacy of the transfusion, the mean total Hb mass will be calculated based on Nadler's formula for blood volume, as follows:

$$For\ males\ = (0.3669 \times height(m)^3 + 0.03219 \times body\ weight(kg) + 0.6041) \times mean\ Hb\ (g/L)$$

$$For\ females\ = (0.3561 \times height(m)^3 + 0.03308 \times body\ weight(kg) + 0.1833) \times mean\ Hb\ (g/L)$$

Mean Hemoglobin calculated as the sum of the values of the mean hemoglobin between two measurements multiply by the $\times \frac{(days\ between\ 2\ measurement)}{(total\ observation\ period)}$

Analyses

Different analysis will be evaluated as per standard follow up protocols for these patients. Samples for further exploratory analyses will be collected and stored

Samples collection and timepoints

Visit Week:	**Baseline Period**							**Treatment Phase**								**Open-Label Extension Period**
	1						**13**	**14**							**38**	**Till LPLV**
Study Day:	**1**						**85**	**92**							**260**	
Transfusion Day:																
Informed consent								X								
Demographics[2]	X															
Eligibility	X															
Medical and surgical history	X							X							X	
Transfused PRBC cc/kg – units	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Medications	X							X							X	X
Vital signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Hematology, including reticulocytes	X	As per standard evaluation						X		As per standard evaluation					X	X
Serum chemistry	X							X							X	As per standard practice
Liver function tests	X							X							X	As per standard practice
Lipid panel	X							X							X	As per standard practice
Iron panel	X							X							X	Q 3months
QOL								X							X	At last visit

Blood sample for exploratory disease parameters								X								X	At last visit
Adverse events and serious adverse events	X	X (continuous)															

LV: last visit with Hemanext
LPLV: Last patient last visit during treatment phase

Hematology includes complete blood count with differential, HCT, Hb, RBC count, absolute reticulocyte count, percent reticulocyte count, MCV, MCH, MCHC, RDW, WBC count with differential, and platelet count.
Serum chemistry includes sodium, potassium, chloride, calcium, magnesium, phosphorus, carbon dioxide or bicarbonate, albumin, total protein, glucose, BUN or urea, creatinine, and uric acid.
Liver function tests include LDH, ALP, ALT, AST, and bilirubin (total, direct, and indirect).
Lipid panel includes total cholesterol, LDL-C, HDL-C, and triglycerides; samples will be collected after an overnight fast. Iron panel may include iron, serum ferritin, total iron binding capacity, or transferrin saturation.

Statistical Method:
For continuous parameters, summary statistics will include the number of subjects, mean, standard deviation, quartiles, minimum and maximum, and the 95% confidence interval of the mean (if appropriate). For categorical parameters, summary statistics will include counts, percentages, and the 95% confidence interval of the percentages (if appropriate) in each category.

Based on analysis on random patients from our unit, the standard deviation (SD) of transfusion burden (annual consumption in cc/kg/year) was estimated at 22.8. The non-inferiority limit was set at 15%. For a power of 90%, 25 participants are required. Assuming a drop-out rate of 10%, the target enrollment will be at least 28 patients.

An interim analysis will be performed when half of the expected enrolled patients (the 15th patient) have reached week 12 of the treatment phase.

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